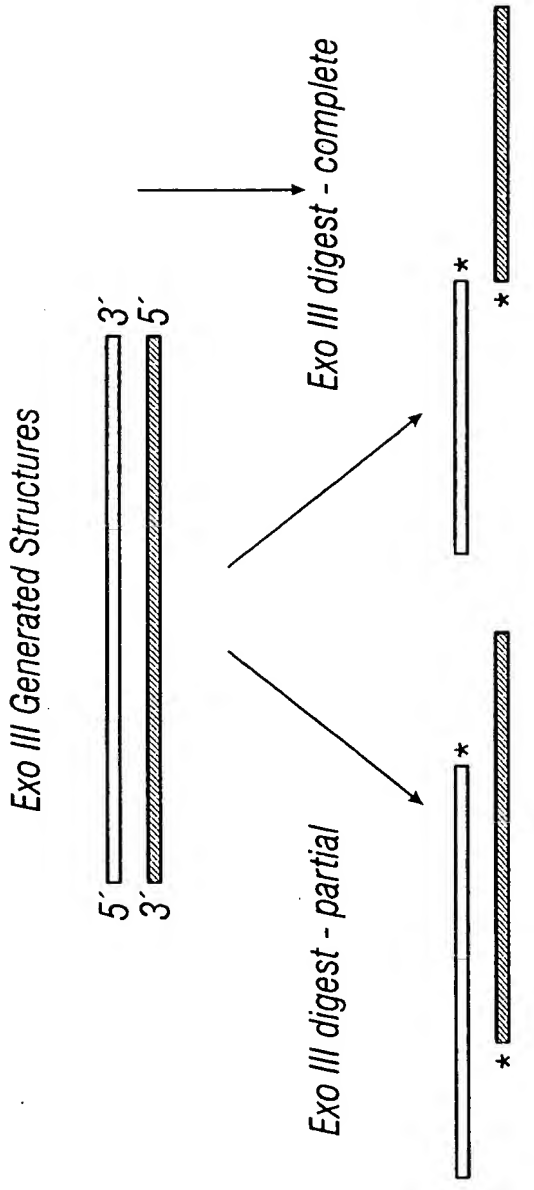


FIG. 1

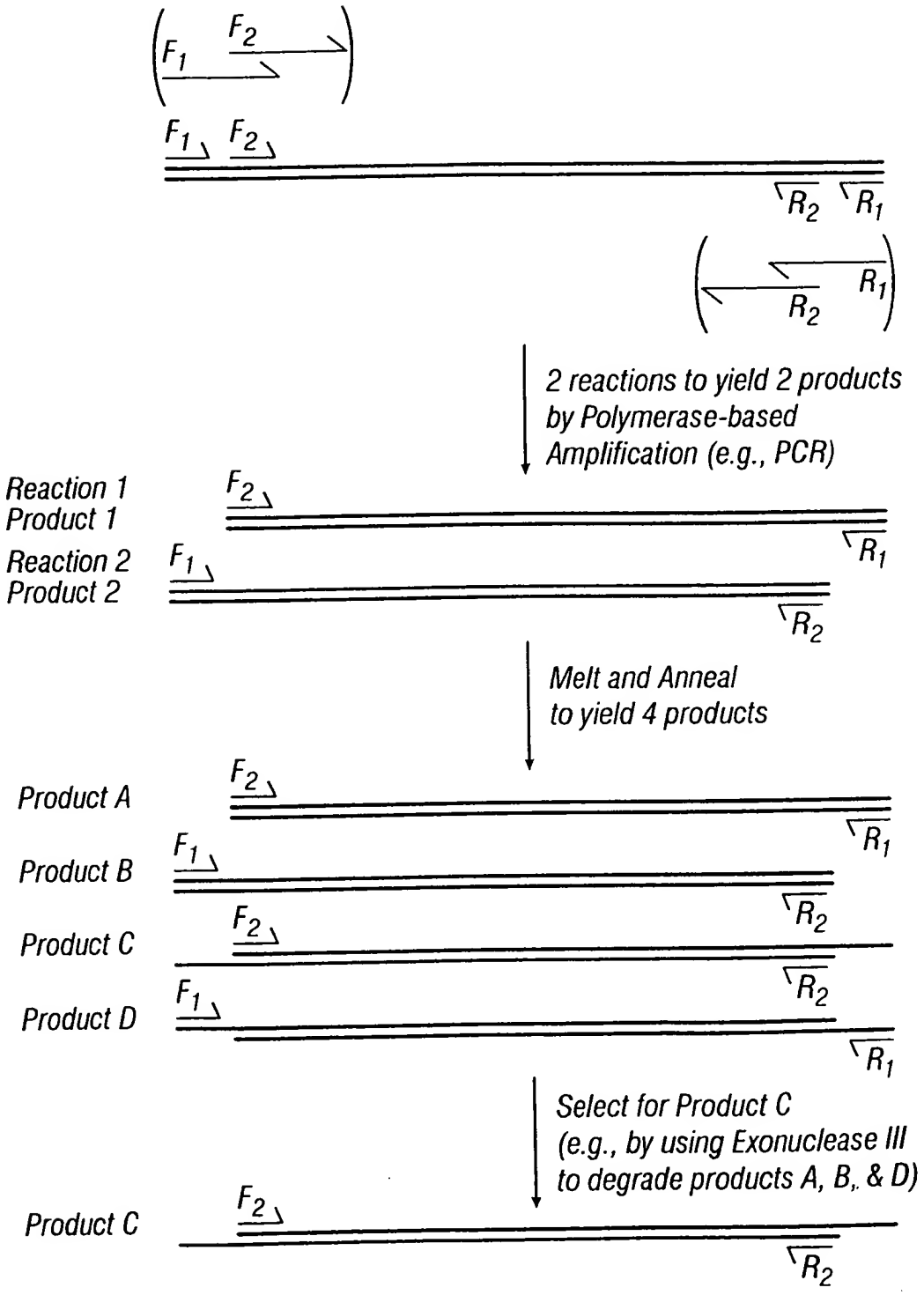

F1
F2
F1
F2
R2
R1
R1
R2
2 reactions to yield 2 products
by Polymerase-based
Amplification (e.g., PCR)
Reaction 1
Product 1
Reaction 2
Product 2
F2
R1
F1
R2
Melt and Anneal
to yield 4 products
Product A
Product B
Product C
Product D
F2
R1
F1
R2
F2
R2
F1
R1
Select for Product C
(e.g., by using Exonuclease III
to degrade products A, B, & D)
Product C
F2
R2

FIG. 2

Panel A.

Panel B.

Panel C.

Panel D.

FIG. 3

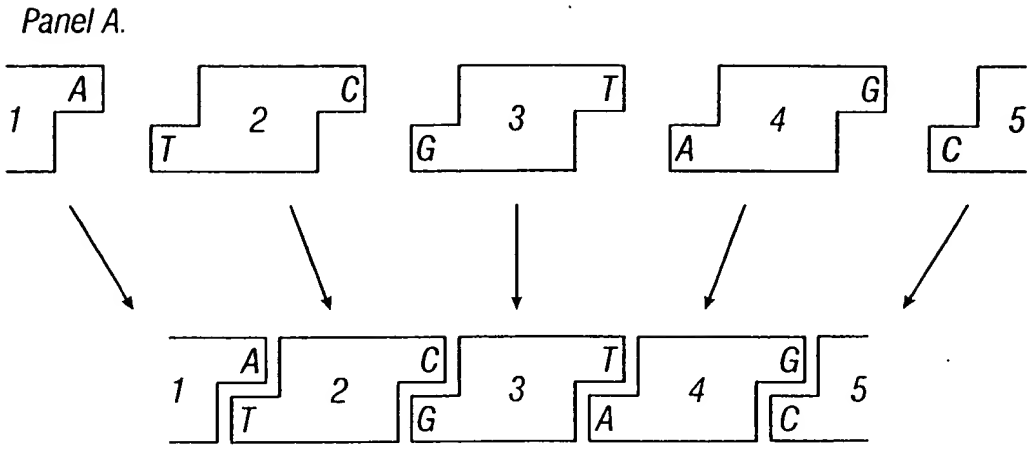


FIG. 4A

Panel C.

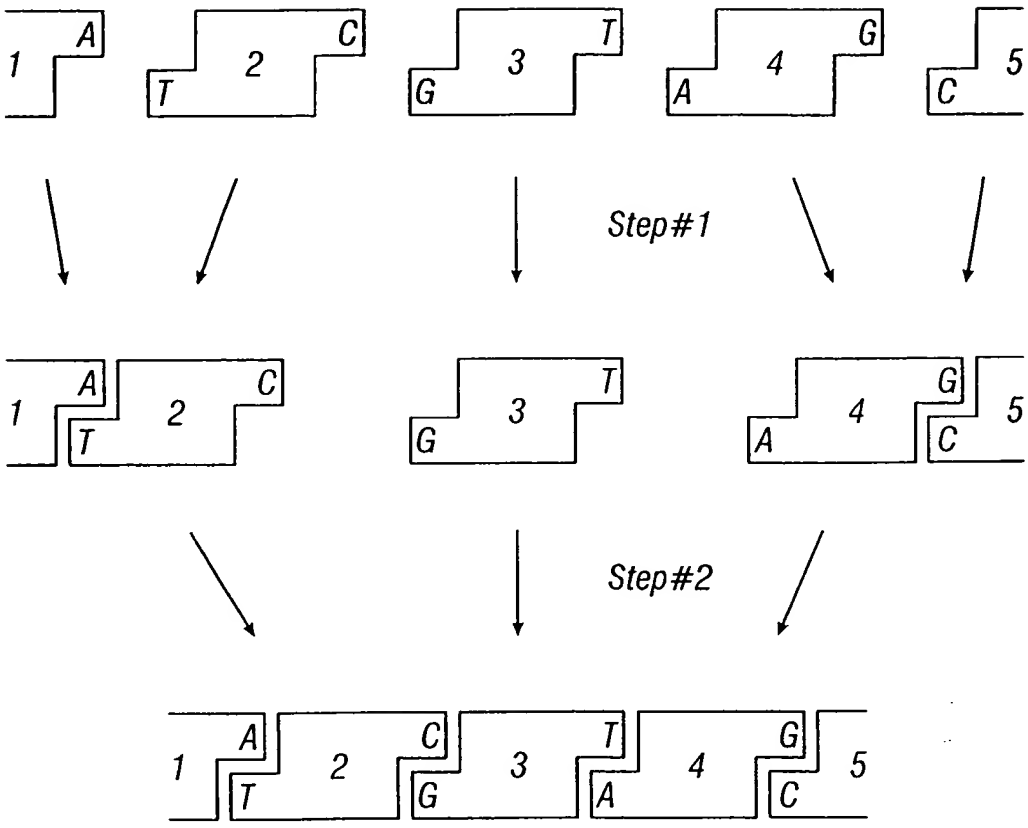


FIG. 4B

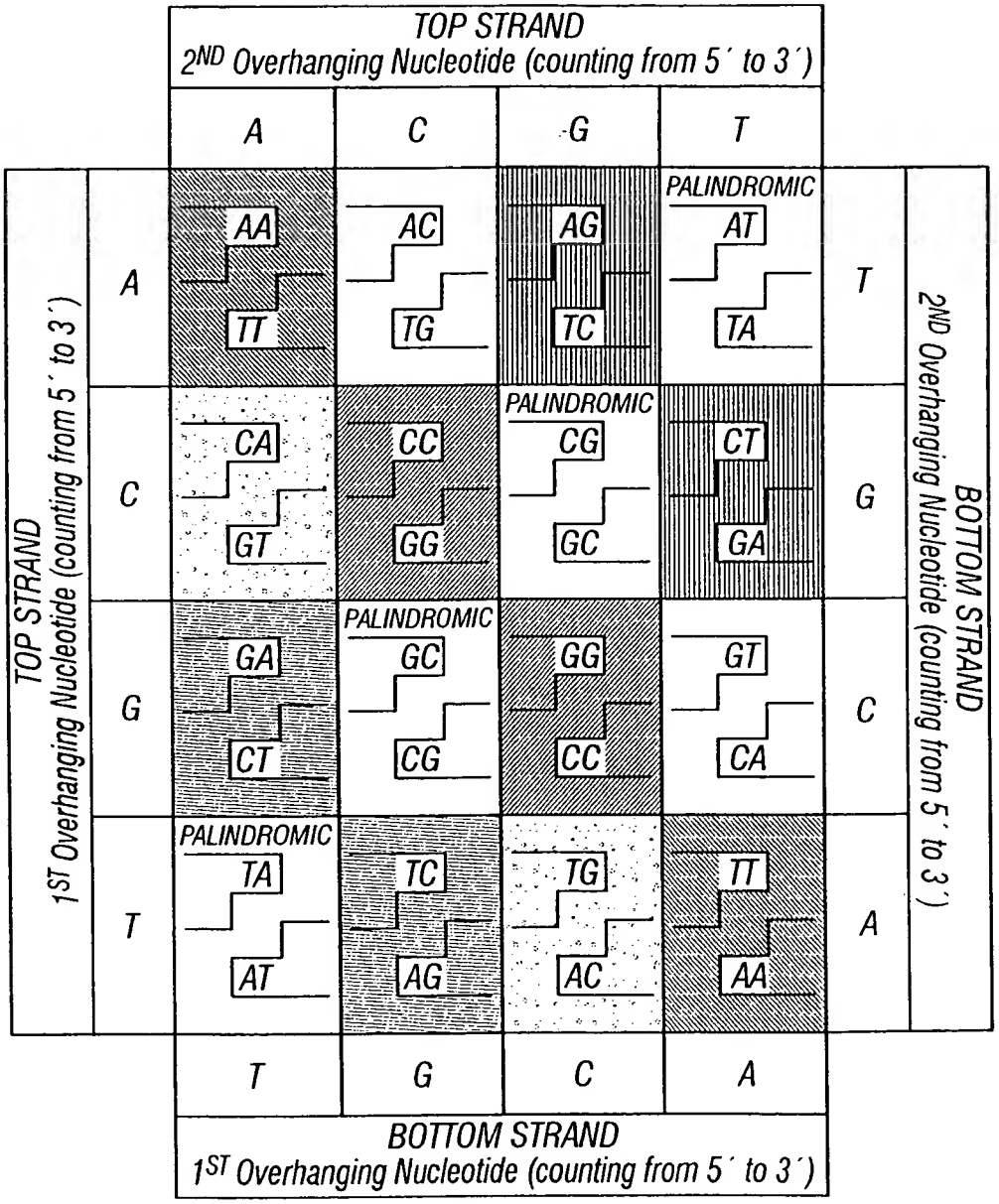

TOP STRAND
2ND Overhanging Nucleotide (counting from 5´ to 3´)
A
C
G
T
TOP STRAND
1ST Overhanging Nucleotide (counting from 5´ to 3´)
BOTTOM STRAND
2ND Overhanging Nucleotide (counting from 5´ to 3´)
A
AA
TT
AC
TG
AG
TC
PALINDROMIC
AT
TA
T
C
CA
GT
CC
GG
PALINDROMIC
CG
GC
CT
GA
G
G
GA
CT
PALINDROMIC
GC
CG
GG
CC
GT
CA
C
T
PALINDROMIC
TA
AT
TC
AG
TG
AC
TT
AA
A
T
G
C
A
BOTTOM STRAND
1ST Overhanging Nucleotide (counting from 5´ to 3´)

FIG. 5

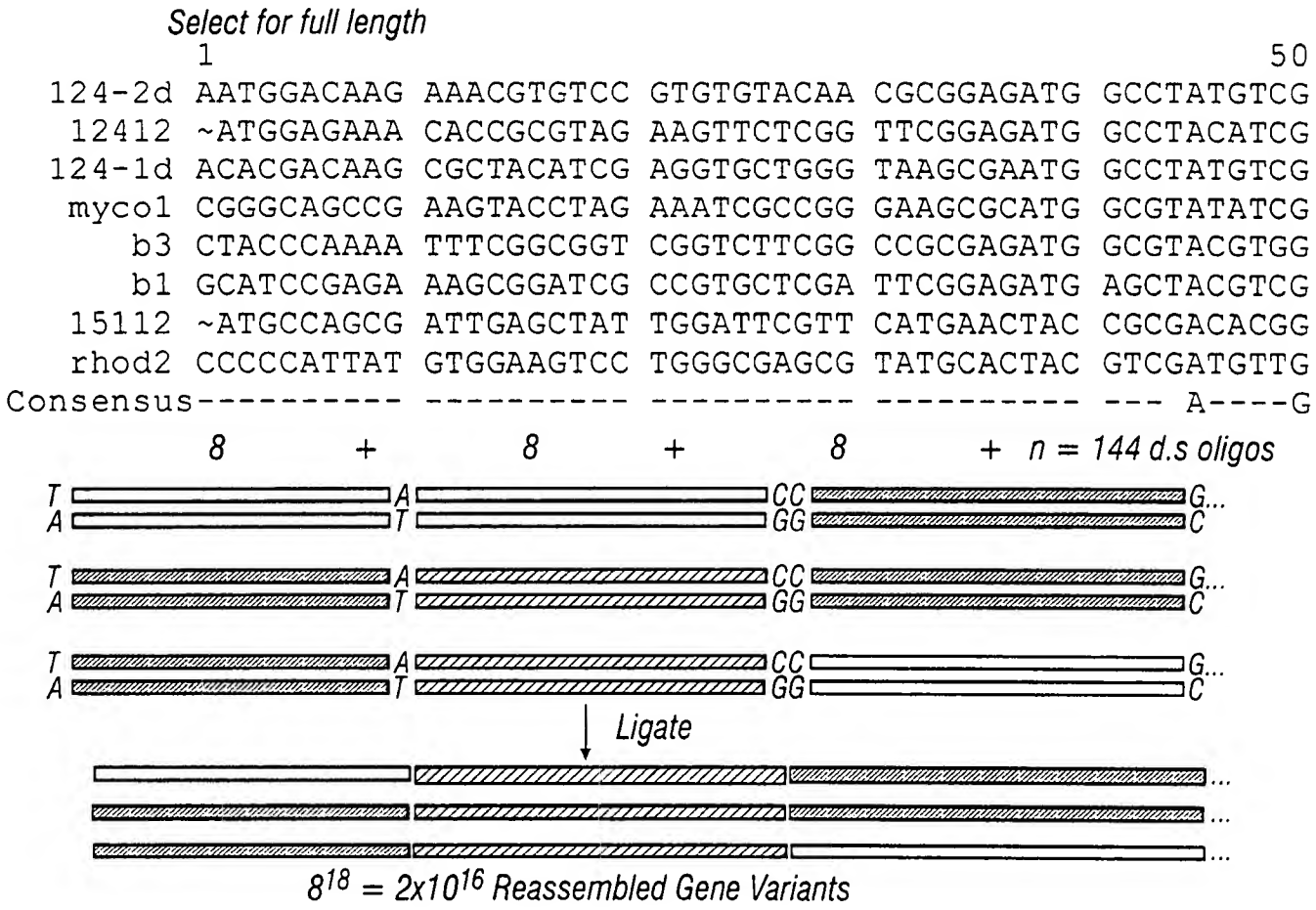

Select for full length
1
50
124-2d AATGGACAAG AAACGTGTCC GTGTGTACAA CGCGGAGATG GCCTATGTCG
12412 ~ATGGAGAAA CACCGCGTAG AAGTTCTCGG TTCGGAGATG GCCTACATCG
124-1d ACACGACAAG CGCTACATCG AGGTGCTGGG TAAGCGAATG GCCTATGTCG
myco1 CGGGCAGCCG AAGTACCTAG AAATCGCCGG GAAGCGCATG GCGTATATCG
b3 CTACCCAAAA TTTCGGCGGT CGGTCTTCGG CCGCGAGATG GCGTACGTGG
b1 GCATCCGAGA AAGCGGATCG CCGTGCTCGA TTCGGAGATG AGCTACGTCG
15112 ~ATGCCAGCG ATTGAGCTAT TGGATTCGTT CATGAACTAC CGCGACACGG
rhod2 CCCCCATTAT GTGGAAGTCC TGGGCGAGCG TATGCACTAC GTCGATGTTG
Consensus---------- ---------- ---------- ---------- --- A----G
8 + 8 + 8 + n = 144 d.s oligos
Ligate
8^18 = 2x10^16 Reassembled Gene Variants

FIG. 6A

```
                                                                  100
ACACGGGCCA GGGTGATTCC GTTCTGTTTC TTCACGGCAA CCCGACGTCG
ACGTGGGAGA GGGCGACCCG ATCGTGTTCC TCCACGGAAA TCCCACGTCG
AGATGGGCGA GGGTGATCCC ATCATTTTCC AACACGGCAA TCCGACCTCA
ACGAAGGCAA GGGTGACGCC ATCGTCTTTC AGCACGGCAA CCCCACGTCG
AAGTGGGACG GGGCGACCCC ATCGTACTCT TGCACGGCAA CCCCACCTCG
ATACCGGCGA GGGAGCGCCG ATCGTGTTCC TTCACGGCAA CCCGACTTCC
GCGTCGGCGA T...CTTCCC GTCGTGTTCC TGCACGGCAA CCCCACGTCG
GACCGCGGGA TGGCACGCCT GTGCTGTTCC TGCACGGTAA CCCGACCTCG
------G--- --------C- -T--T--T-- --CACGG-AA -CC-AC-TC-
```

FIG. 6B

Represents 15% of gene

```
                                                                  150
TCGTATCTGT GGAGGGGCGT AATGCCTTTT GTGACGGACG TCGCCCGATG
TCGTACCTGT GGCGGAACGT GATTCCCCAC GTTGCCGGCT TGGGACGCTG
TCGTACCTGT GGCGCAACAT CATGCCCCAT GTGCAACAGC TCGGTCGCTG
TCTTACTTGT GGCGCAACAT CATGCCGCAC TTGGAAGGGC TGGGCCGGCT
TCGTACCTCT GGCGCAACGT GTTGCCGCAC CTGGCGCCGT TAGGCCGCTG
TCCTATCTTT GGCGCAACAT CATCCCCTAT CTCGCGGATC ACGGCAGATG
TCTCACGTCT GGCGCAACGT GATCCCGCAC GTCGCTGGCC AGCACCGGTG
TCCTACCTGT GGCGCAACAT CATCCCGCAT GTAGCACCGA GTCATCGGTG
TC--A--T-T GG-G---C-T --T-CC---- -T-------- ------G---
```

FIG. 6C


NcoI
CCGT
150am13_00 cATGATGCACG GCGATATTTC ATCGAGCAAT GACACGGTCG GCGTTGCCGT
150AM7_001 cATGCATCACG GCGACATTTC ATCGAGCAAT GACACGGTCG GCGTTGCCGT
431am7_002 cATGAGACACG GAGATATCTC CAGCAGCAAC GATTGCGTGG GCGTGGCCGT
GAG GT
150am13_00 CGTGAACTAC AAGATGCCTC GCCTTCATAC CAAGGCGGAG GTTTTAGCGA
150AM7_001 CGTGAACTAC AAGATGCCGC GGCTTCACAC CAAGGCTGAG GTGCTGGCCA
431am7_002 CGTGAACTAC AAGATGCCGC GGCTGCATAC CCGCGCGGAG GTGATGGAGA
CGG
150am13_00 ACGCCAGAAA GATCGGCGAG ATGATCGTCG GCATGAAGAC CGGCCTGCCC
150AM7_001 ACTGCCGCAA GATCGCCGAC ATGCTGGTCG GCATGAAGAG CGGCCTGCCG
431am7_002 ACGCCCGCAA GATCGCCGAC ATGGTCGTGG GCATGAAGCG CGGCCTGCCC
CCACG
150am13_00 GGAATGGATC TGGTGATCTT CCCGGAATAT TCGACCCACG GCATCATGTA
150AM7_001 GGAATGGATC TGGTGATCTT CCCGGAATAT TCCACCCACG GCATCATGTA
431am7_002 GGCATGGACC TGGTCATCTT CCCCGAGTAC TCCACCCACG GCATCATGTA
CCC GG
150am13_00 CGACTCCAAG GAAATGTACG ATACCGCGTC CGTCGTGCCC GGCGAGGAGA
150AM7_001 CGACTCCAAG GAGATGTACG ACACGGCGTC GACGGTGCCG GGTGAAGAGA
431am7_002 CGACGCCAAG GAAATGTACG AAACCGCTTC GGCCATTCCG GGCGAAGAGA
G GGG
150am13_00 CCGAGATTTT TGCCGAAGCC TGCCGCAAGG CGAAAGTCTG GGGCGTGTTC
150AM7_001 CCGAGATTTT CGCCGAGGCC TGCCGCAAGG CCAAGGTCTG GGGCGTGTTC
431am7_002 CTGCTGTGTT CGCCGACGCC TGCCGCAAGG CCAACGTATG GGGCGTGTTT

FIG. 7A

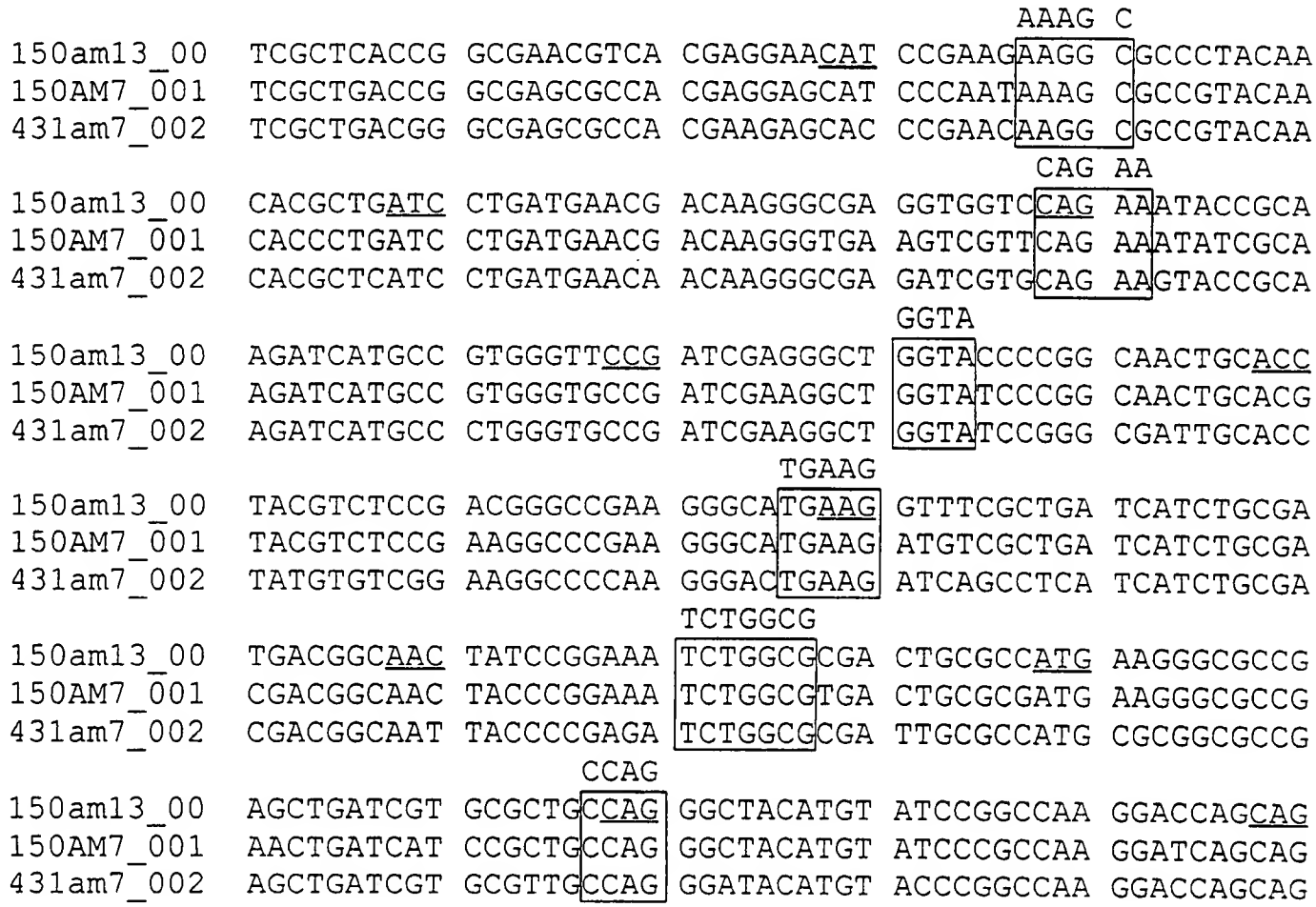

AAAG C
150am13_00 TCGCTCACCG GCGAACGTCA CGAGGAACAT CCGAAGAAGG CGCCCTACAA
150AM7_001 TCGCTGACCG GCGAGCGCCA CGAGGAGCAT CCCAATAAAG CGCCGTACAA
431am7_002 TCGCTGACGG GCGAGCGCCA CGAAGAGCAC CCGAACAAGG CGCCGTACAA
CAG AA
150am13_00 CACGCTGATC CTGATGAACG ACAAGGGCGA GGTGGTCCAG AAATACCGCA
150AM7_001 CACCCTGATC CTGATGAACG ACAAGGGTGA AGTCGTTCAG AAATATCGCA
431am7_002 CACGCTCATC CTGATGAACA ACAAGGGCGA GATCGTGCAG AAGTACCGCA
GGTA
150am13_00 AGATCATGCC GTGGGTTCCG ATCGAGGGCT GGTACCCCGG CAACTGCACC
150AM7_001 AGATCATGCC GTGGGTGCCG ATCGAAGGCT GGTATCCCGG CAACTGCACG
431am7_002 AGATCATGCC CTGGGTGCCG ATCGAAGGCT GGTATCCGGG CGATTGCACC
TGAAG
150am13_00 TACGTCTCCG ACGGGCCGAA GGGCATGAAG GTTTCGCTGA TCATCTGCGA
150AM7_001 TACGTCTCCG AAGGCCCGAA GGGCATGAAG ATGTCGCTGA TCATCTGCGA
431am7_002 TATGTGTCGG AAGGCCCCAA GGGACTGAAG ATCAGCCTCA TCATCTGCGA
TCTGGCG
150am13_00 TGACGGCAAC TATCCGGAAA TCTGGCGCGA CTGCGCCATG AAGGGCGCCG
150AM7_001 CGACGGCAAC TACCCGGAAA TCTGGCGTGA CTGCGCGATG AAGGGCGCCG
431am7_002 CGACGGCAAT TACCCCGAGA TCTGGCGCGA TTGCGCCATG CGCGGCGCCG
CCAG
150am13_00 AGCTGATCGT GCGCTGCCAG GGCTACATGT ATCCGGCCAA GGACCAGCAG
150AM7_001 AACTGATCAT CCGCTGCCAG GGCTACATGT ATCCCGCCAA GGATCAGCAG
431am7_002 AGCTGATCGT GCGTTGCCAG GGATACATGT ACCCGGCCAA GGACCAGCAG

FIG. 7B

```
                                    GC
150am13_00  GTCATCATGG CGAAGGCGAT GGCGTGGGCG AATAATTGTT ACGTCGCGGT
150AM7_001  GTGCTGATGG CGAAAGCAAT GGCCTGGGCC AACAACGTTT ATGTCGCGGT
431am7_002  GTCATGGTGT CCAAGGCCAT GGCGTGGATG AACAACGTCT ACGTGGCGGT
                          GGGCTTCG
150am13_00  TTCCAATGCC GCGGGCTTCG ATGGCGTCTA TTCGTATTTC GGCCACTCGG
150AM7_001  CGCCAATGCC TCGGGCTTCG ACGGCGTCTA CTCGTATTTC GGCCATTCGG
431am7_002  GGCCAATGCC GCGGGCTTCG ACGGCGTGTA TTCCTACTTC GGCCATTCGG
                        TTCGA
150am13_00  CGATCATCGG CTTCGATGGC CGCACGCTCG GCGAATGCGG CGAGGAAGAA
150AM7_001  CGATCATCGG CTTCGACGGC CGTACCCTCG GCGAATGCGG CGAGGAGGAT
431am7_002  CCATCATCGG CTTCGACGGC CGCACGCTGG GCGAATGCGG TGAAGAAGAC
                      C AGTA
150am13_00  TACGGCATCC AGTATGCCCA GCTTTCGAAG ATGCTGATCC GCGACGCCCG
150AM7_001  TATGGCATCC AGTATGCCGC CATCTCCAAG TCGCTGATCC GCGACGCGCG
431am7_002  ATGGGCGTGC AGTACGCCGA GCTCTCCACC AGCCTGATCC GCGACGCGCG
                        CAATC
150am13_00  CCGCACCGGA CAATCGGAAA ACCATCTCTT CAAGCTGGTG CATCGTGGCT
150AM7_001  CCGCACCGGC CAATCGGAAA ACCATCTCTT CAAGCTGGTG CACCGTGGCT
431am7_002  CAAGAACATG CAGTCGCAGA ACCACTTGTT CAAGCTGGTG CACCGCGGCT
                        GATCAA
150am13_00  ACACCGGGTT GATCAACTCC GGCGAGGGCG ACCGCGGTCT CGCGGCCTGT
150AM7_001  ACACCGGCAT GATCAATTCC GGCGAGGGCG ACCGCGGTGT CGCGGCTTGC
431am7_002  ACACCGGCAA GATCAATTCC GGCGAAGAGG CCACCGGCGT CGCGGCATGC
```

FIG. 7C

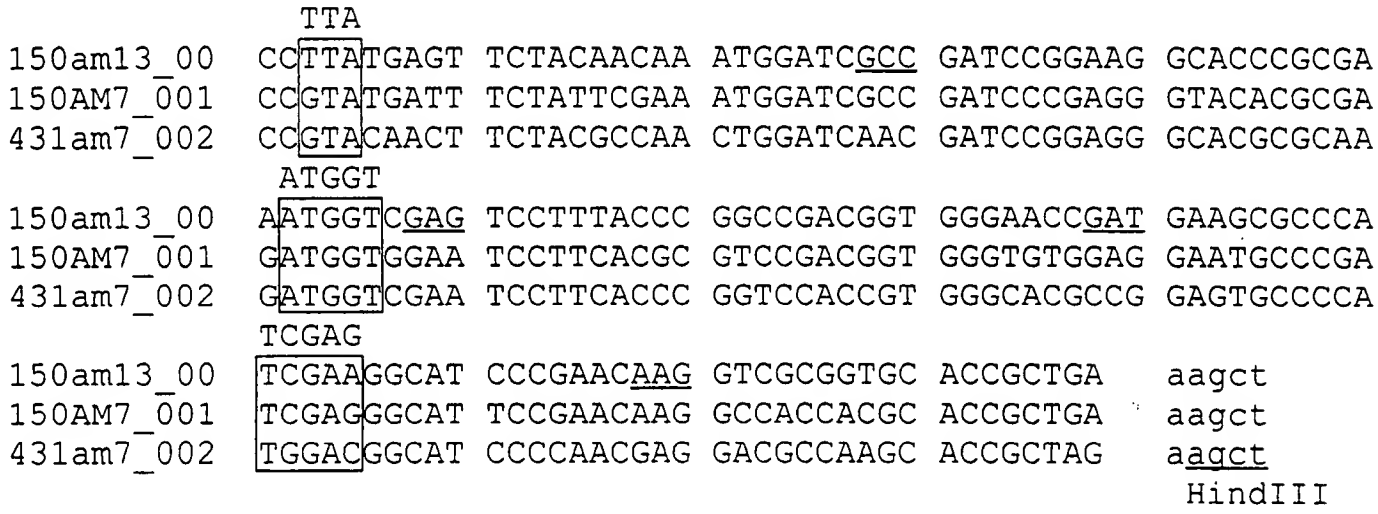

TTA
150am13_00 CCTTATGAGT TCTACAACAA ATGGATCGCC GATCCGGAAG GCACCCGCGA
150AM7_001 CCGTATGATT TCTATTCGAA ATGGATCGCC GATCCCGAGG GTACACGCGA
431am7_002 CCGTACAACT TCTACGCCAA CTGGATCAAC GATCCGGAGG GCACGCGCAA
ATGGT
150am13_00 AATGGTCGAG TCCTTTACCC GGCCGACGGT GGGAACCGAT GAAGCGCCCA
150AM7_001 GATGGTGGAA TCCTTCACGC GTCCGACGGT GGGTGTGGAG GAATGCCCGA
431am7_002 GATGGTCGAA TCCTTCACCC GGTCCACCGT GGGCACGCCG GAGTGCCCCA
TCGAG
150am13_00 TCGAAGGCAT CCCGAACAAG GTCGCGGTGC ACCGCTGA aagct
150AM7_001 TCGAGGGCAT TCCGAACAAG GCCACCACGC ACCGCTGA aagct
431am7_002 TGGACGGCAT CCCCAACGAG GACGCCAAGC ACCGCTAG aagct
HindIII

FIG. 7D

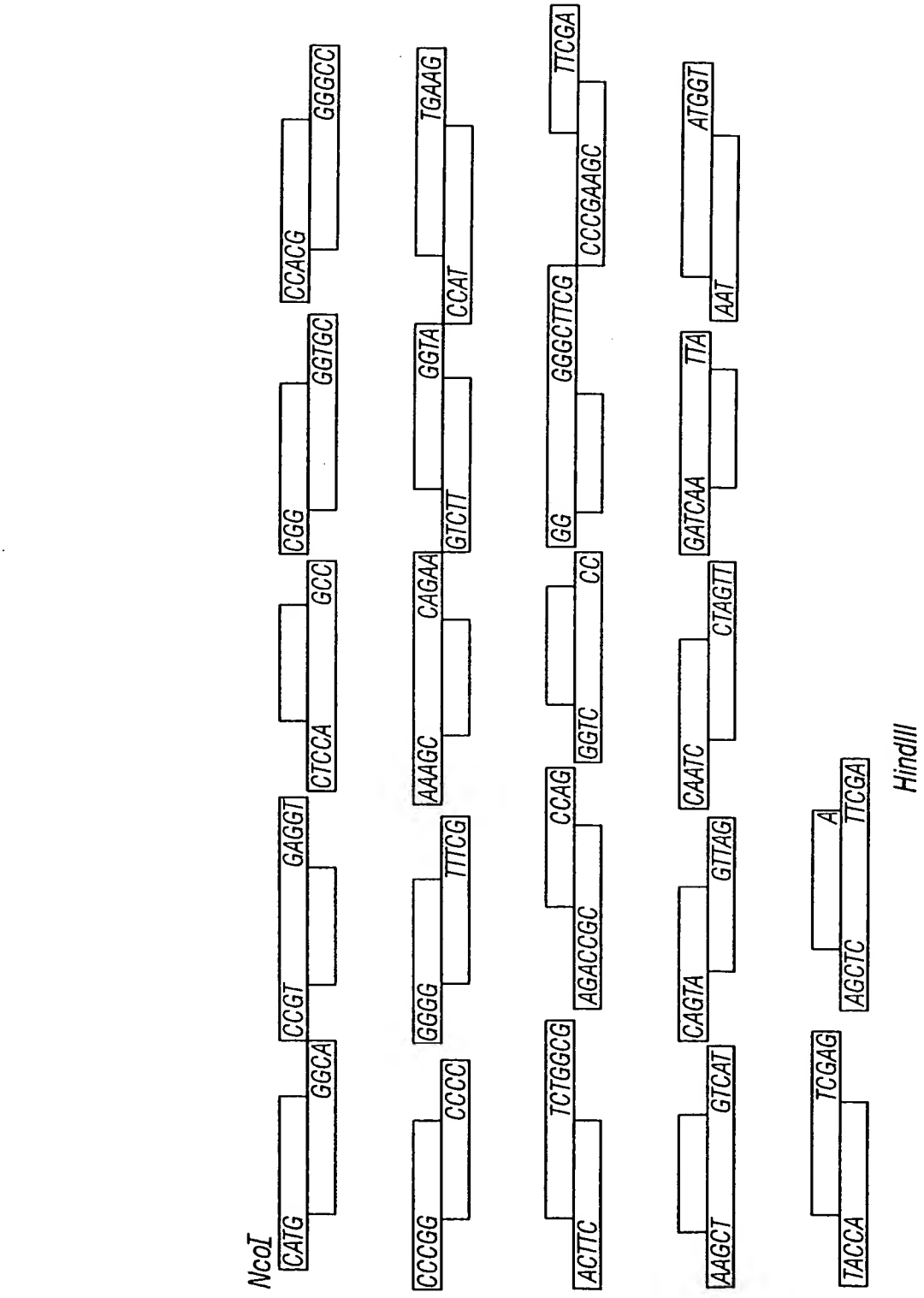

NcoI
CATG
GGCA
CCGT
GAGGT
CTCCA
GCC
CGG
GGTGC
CCACG
GGGCC
CCCGG
CCCC
GGGG
TTTCG
AAAGC
CAGAA
GTCTT
GGTA
CCAT
TGAAG
ACTTC
TCTGGCG
AGACCGC
CCAG
GGTC
CC
GG
GGGCTTCG
CCCGAAGC
TTCGA
AAGCT
GTCAT
CAGTA
GTTAG
CAATC
CTAGTT
GATCAA
TTA
AAT
ATGGT
TACCA
TCGAG
AGCTC
A
TTCGA
HindIII

FIG. 8

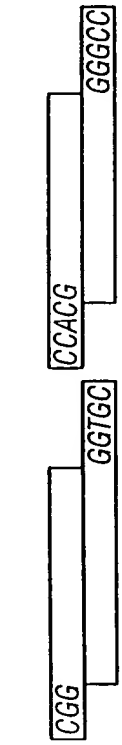
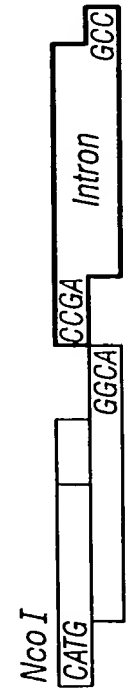

CCACG
GGGCC
CGG
GGTGC

FIG. 9


Nco I
CATG
GGCA
CCGA
Intron
GCC

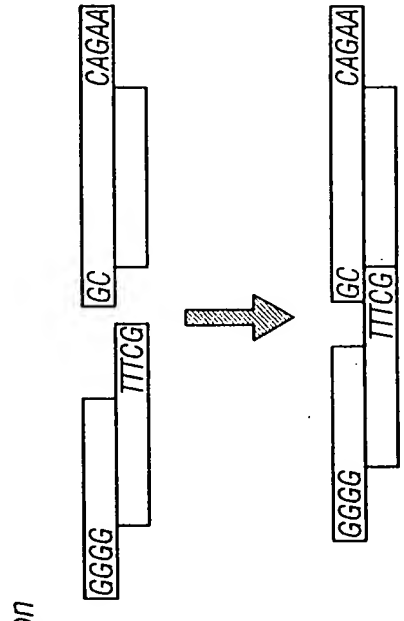

Gap Ligation
GGGG
TTTCG
GC
CAGAA
GGGG
TTTCG
GC
CAGAA

FIG. 10

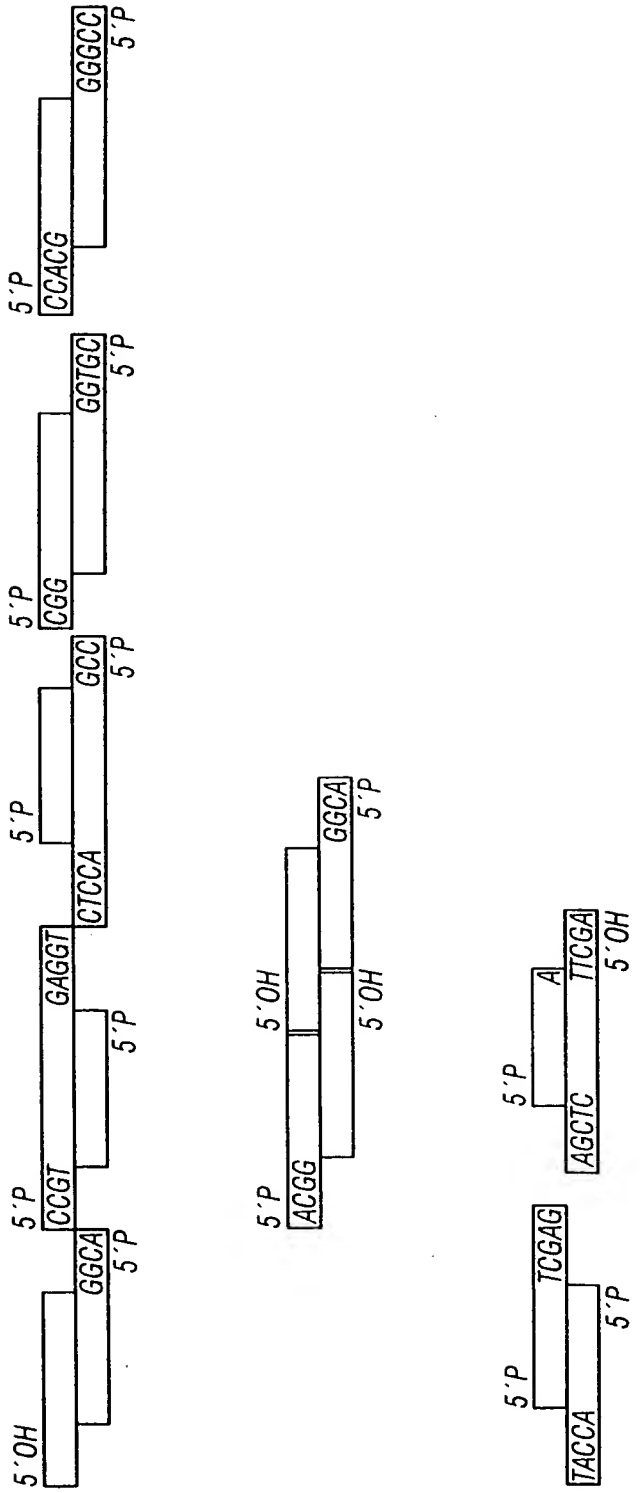

5'OH
GGCA
5'P
5'P
CCGT
GAGGT
CTCCA
GCC
5'P
5'P
5'P
CGG
GGTGC
5'P
5'P
CCACG
GGGCC
5'P
5'OH
5'P
ACGG
GGCA
5'P
5'OH
5'P
TACCA
TCGAG
5'P
5'P
AGCTC
A
TTCGA
5'OH

FIG. 11

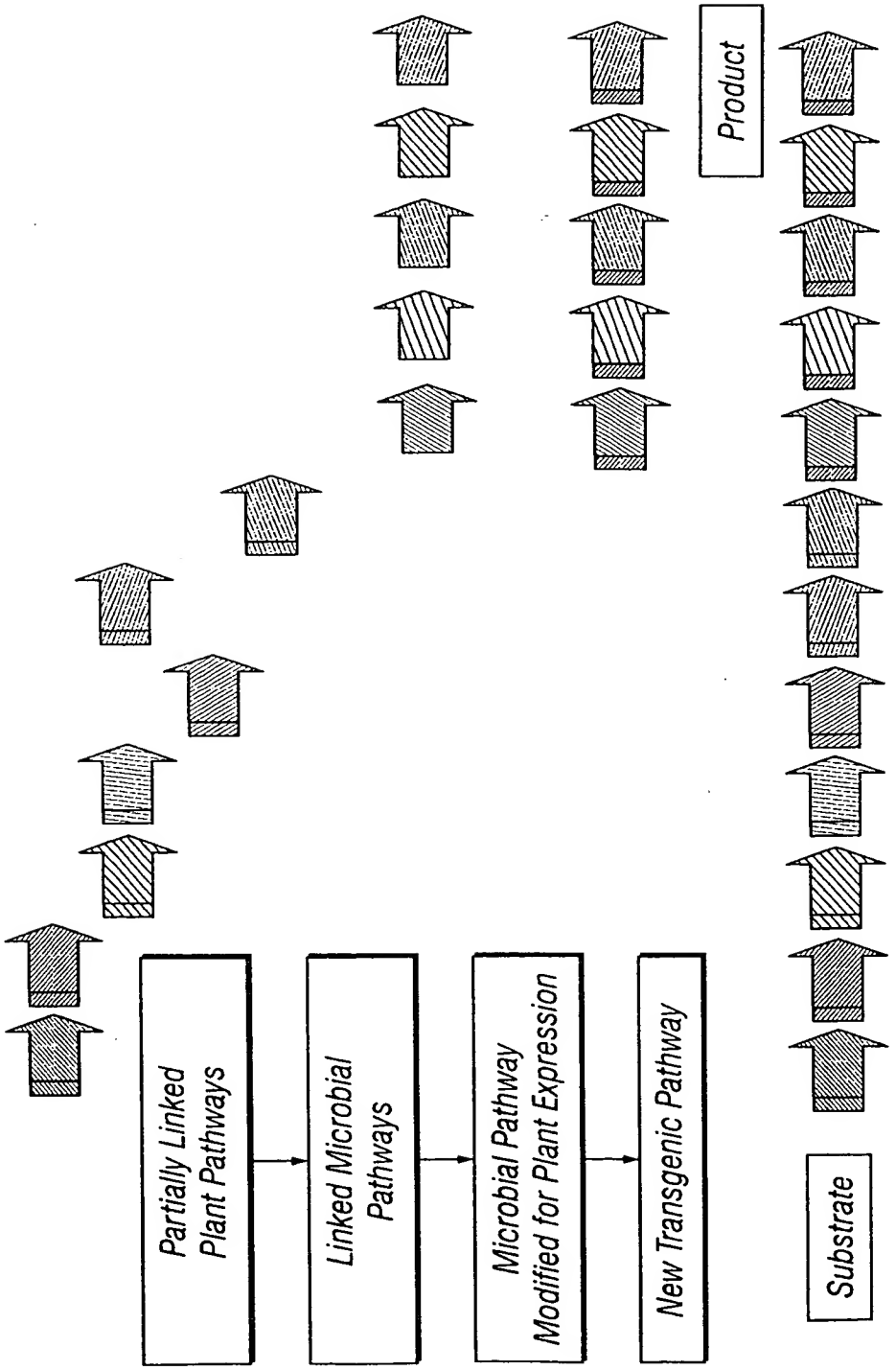

Partially Linked Plant Pathways
Linked Microbial Pathways
Microbial Pathway Modified for Plant Expression
New Transgenic Pathway
Substrate
Product

FIG. 12

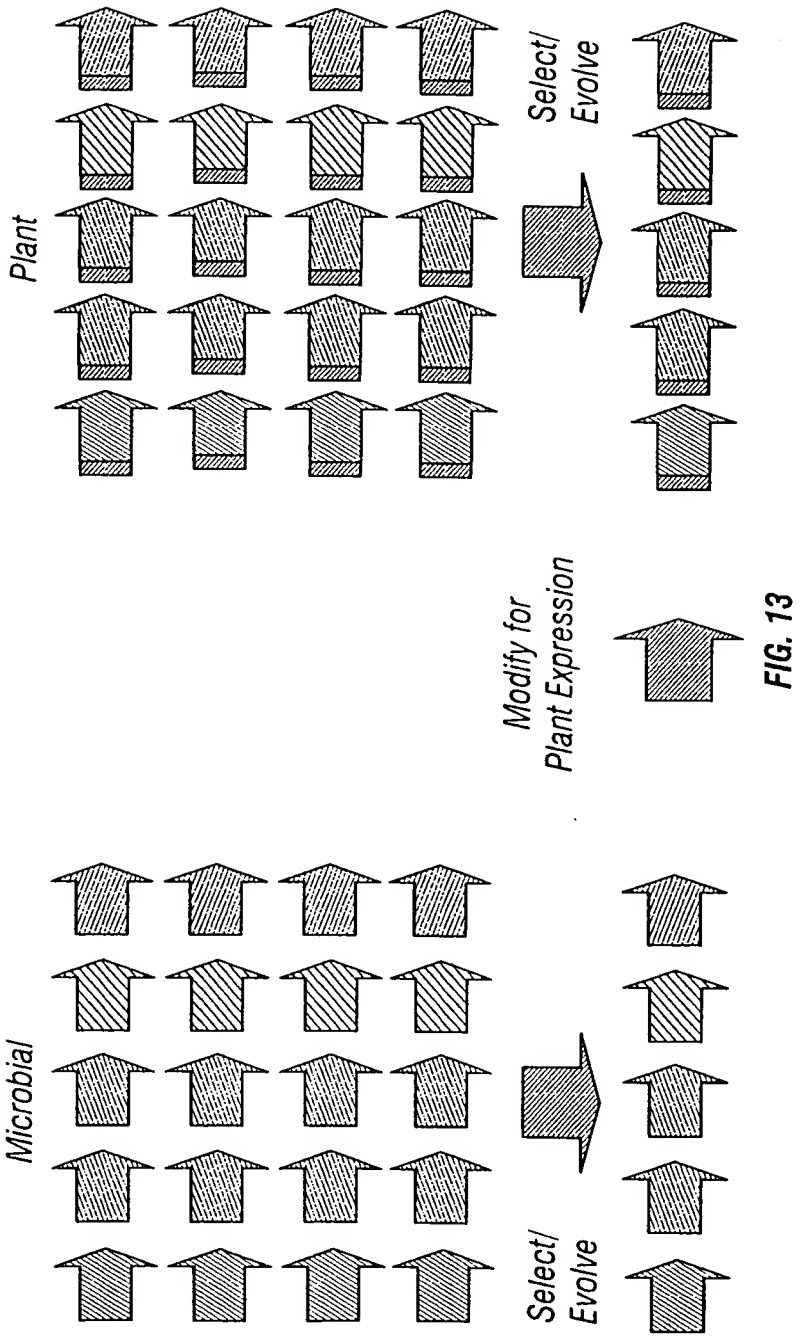

Plant
Select/
Evolve
Modify for
Plant Expression
Microbial
Select/
Evolve

FIG. 13

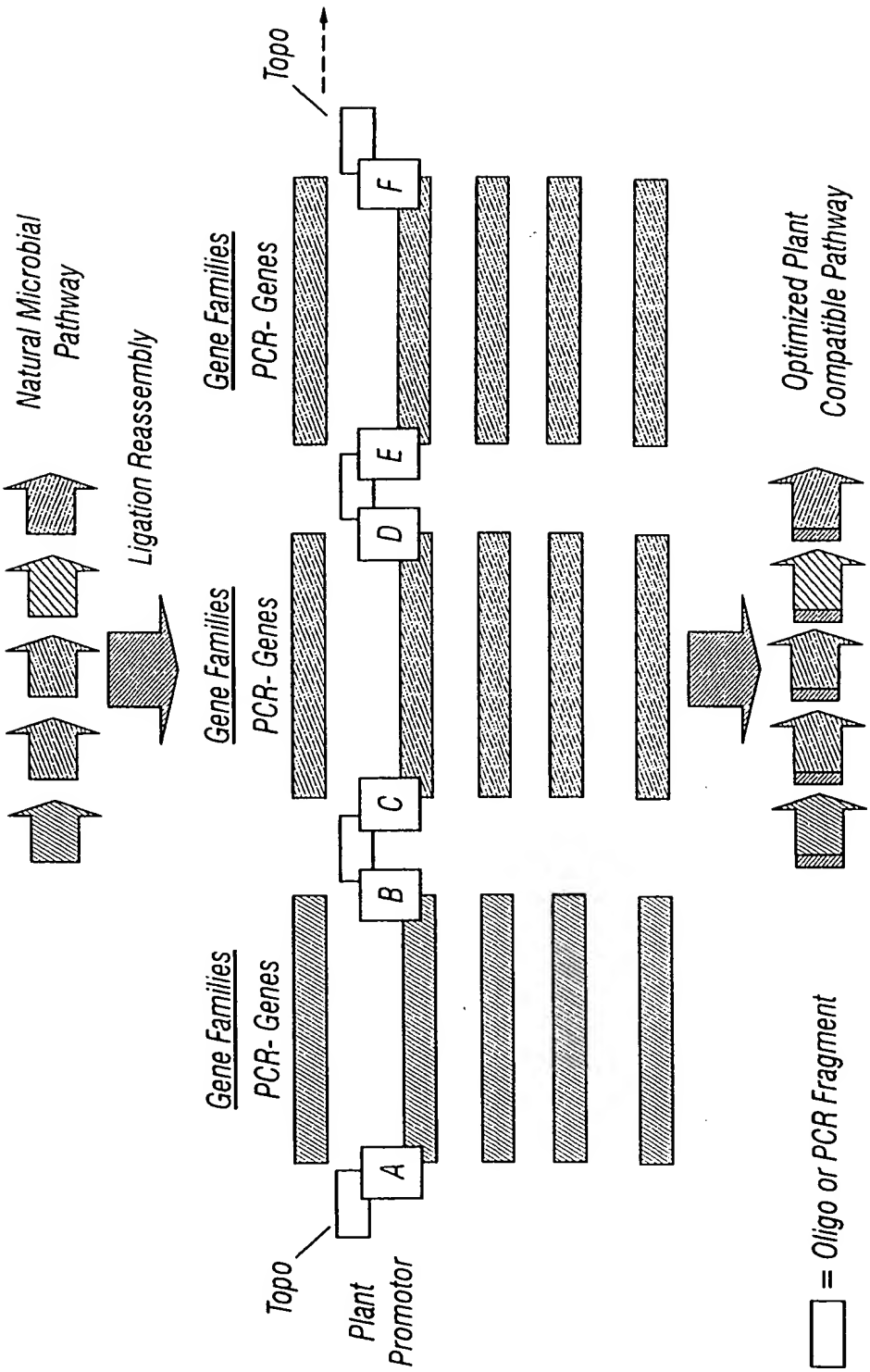

Natural Microbial Pathway
Ligation Reassembly
Gene Families
PCR- Genes
Gene Families
PCR- Genes
Gene Families
PCR- Genes
Topo
Plant Promotor
A
B
C
D
E
F
Topo
Optimized Plant Compatible Pathway
= Oligo or PCR Fragment

FIG. 14

FIG. 15

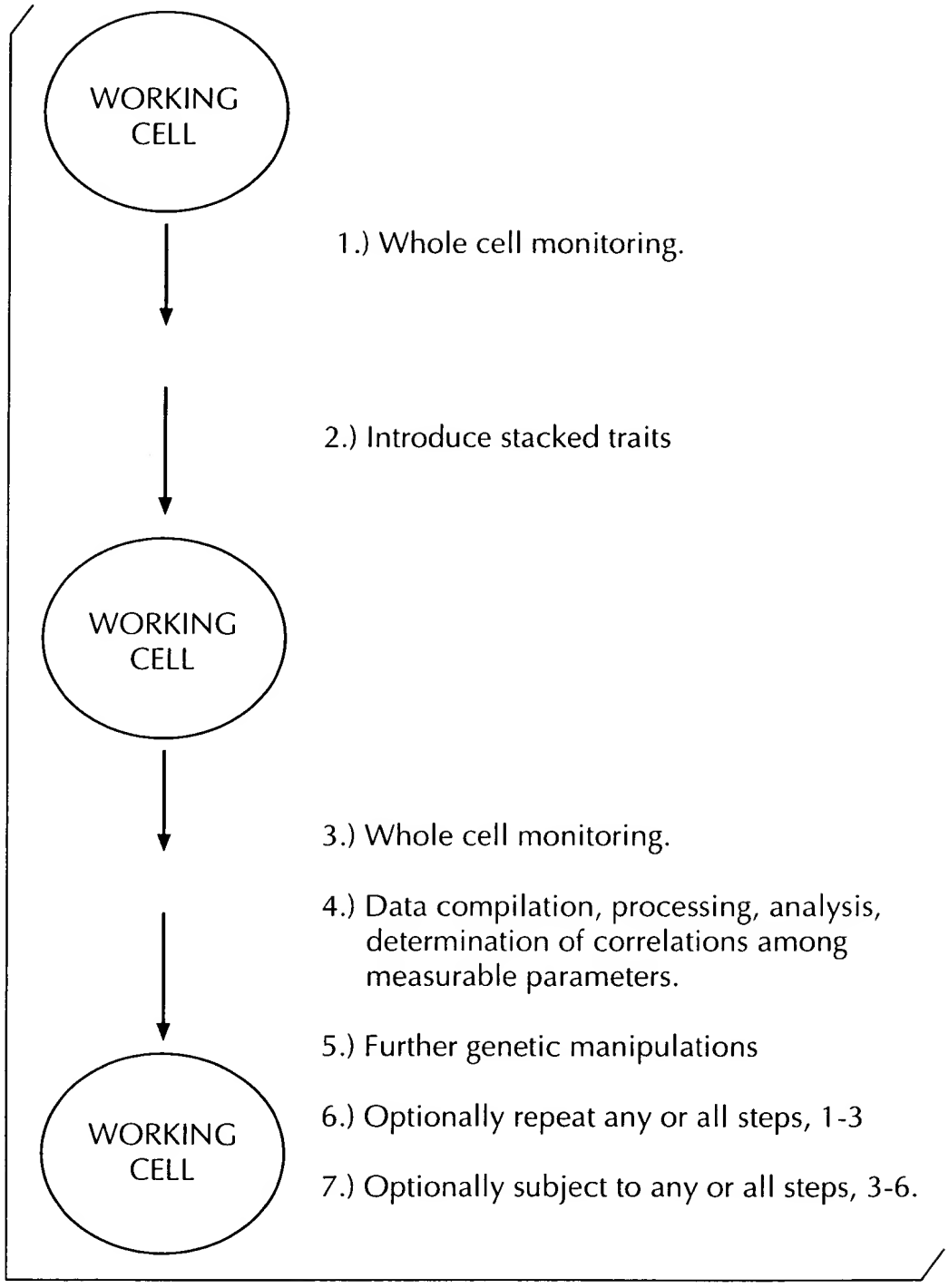

WORKING CELL
1.) Whole cell monitoring.
2.) Introduce stacked traits
WORKING CELL
3.) Whole cell monitoring.
4.) Data compilation, processing, analysis, determination of correlations among measurable parameters.
5.) Further genetic manipulations
WORKING CELL
6.) Optionally repeat any or all steps, 1-3
7.) Optionally subject to any or all steps, 3-6.

FIG. 16

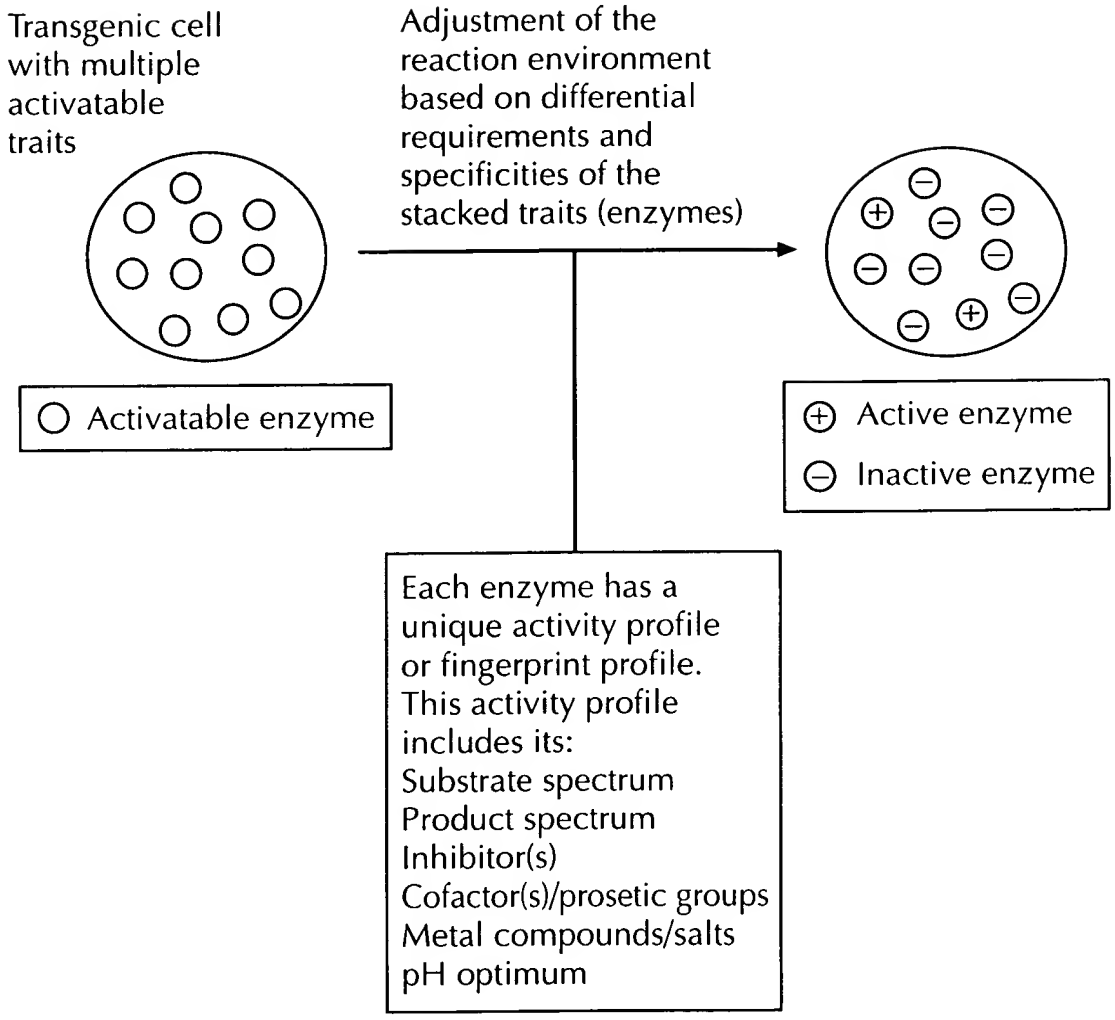

Transgenic cell with multiple activatable traits
Adjustment of the reaction environment based on differential requirements and specificities of the stacked traits (enzymes)
Activatable enzyme
Active enzyme
Inactive enzyme
Each enzyme has a unique activity profile or fingerprint profile. This activity profile includes its:
Substrate spectrum
Product spectrum
Inhibitor(s)
Cofactor(s)/prosetic groups
Metal compounds/salts
pH optimum

FIG. 17

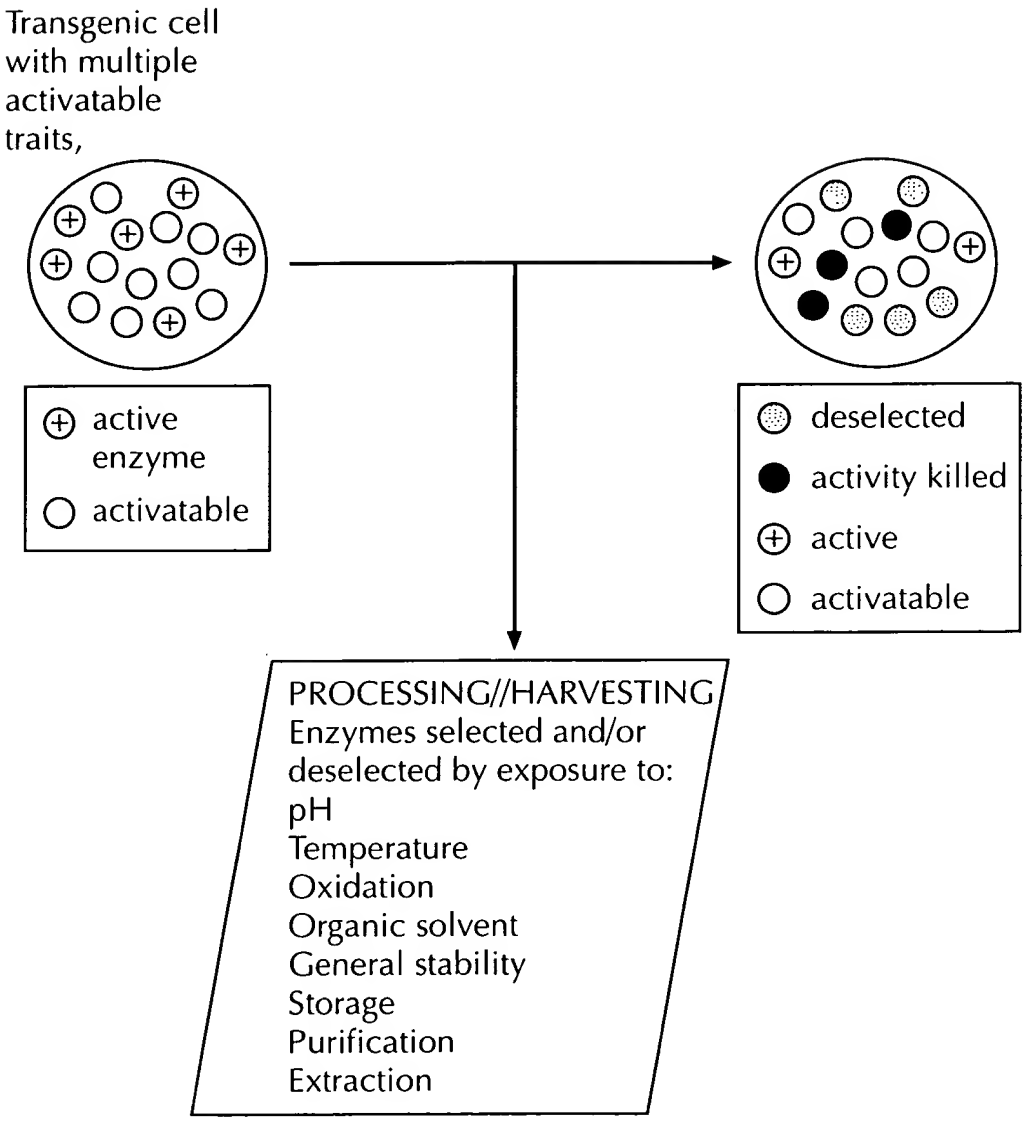

Transgenic cell with multiple activatable traits,
⊕ active enzyme
○ activatable
deselected
activity killed
⊕ active
○ activatable
PROCESSING//HARVESTING
Enzymes selected and/or deselected by exposure to:
pH
Temperature
Oxidation
Organic solvent
General stability
Storage
Purification
Extraction

FIG. 18

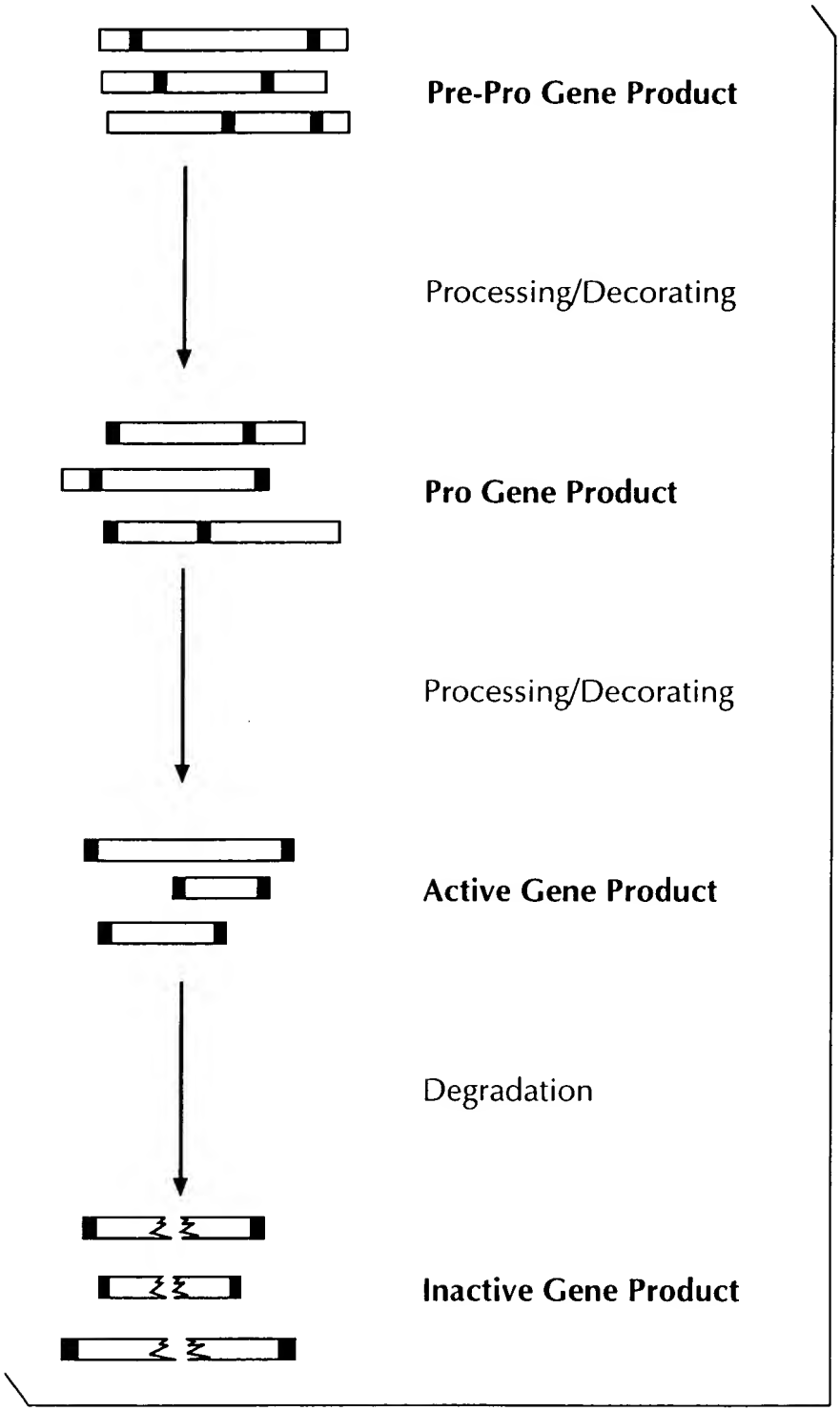

Pre-Pro Gene Product
Processing/Decorating
Pro Gene Product
Processing/Decorating
Active Gene Product
Degradation
Inactive Gene Product

FIG. 19

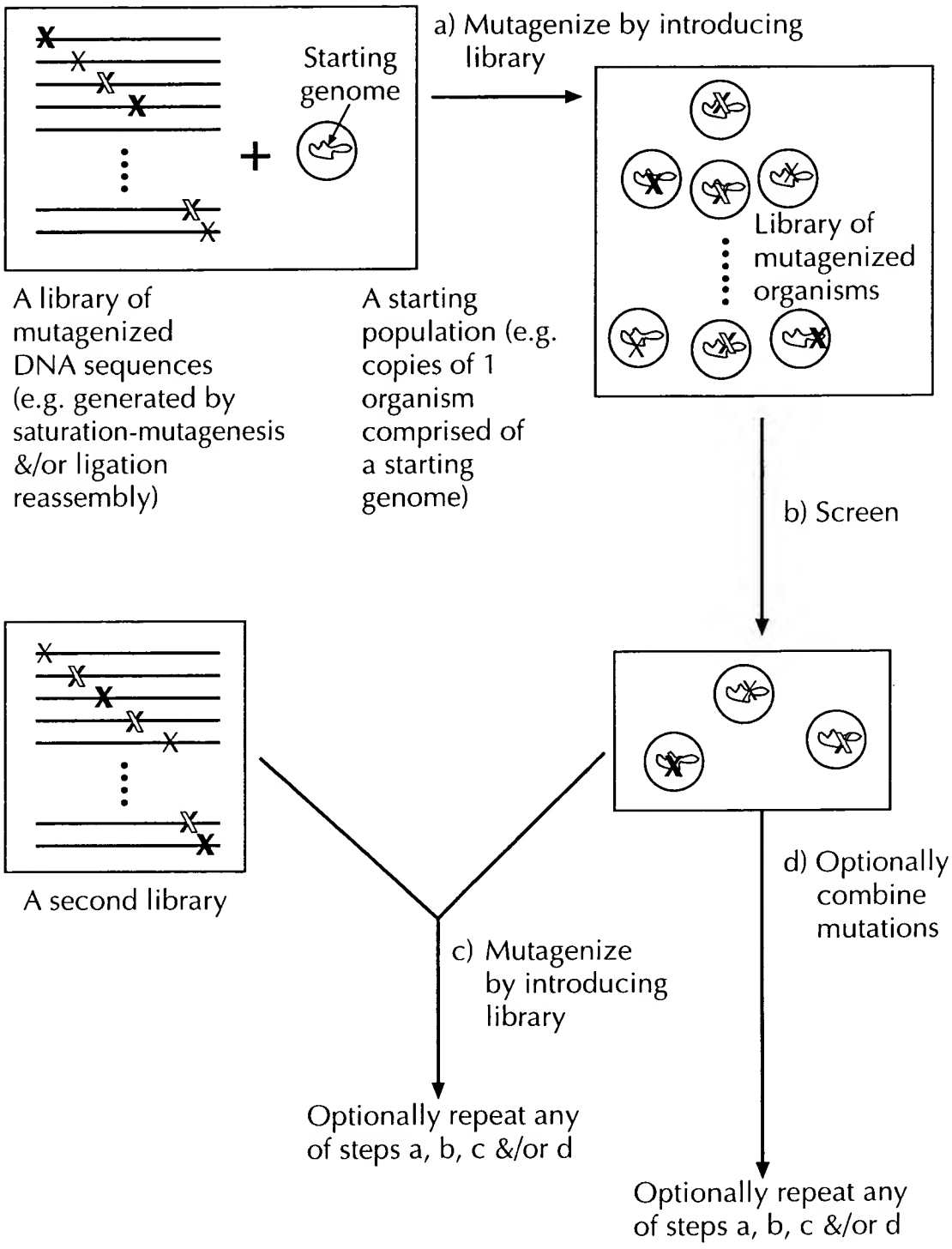
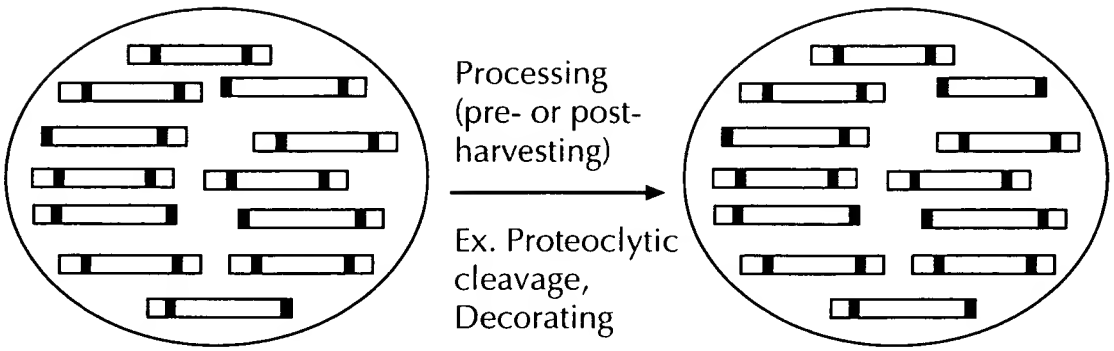

Starting genome
a) Mutagenize by introducing library
Library of mutagenized organisms
A library of mutagenized DNA sequences (e.g. generated by saturation-mutagenesis &/or ligation reassembly)
A starting population (e.g. copies of 1 organism comprised of a starting genome)
b) Screen
A second library
c) Mutagenize by introducing library
d) Optionally combine mutations
Optionally repeat any of steps a, b, c &/or d
Optionally repeat any of steps a, b, c &/or d

FIG. 20



Inactive precursor gene products (ex. pre-pro hormones, pro-hormones pre-pro proteins, or pro-proteins).

LEGEND:

pre-pro

pro

active

FIG. 21

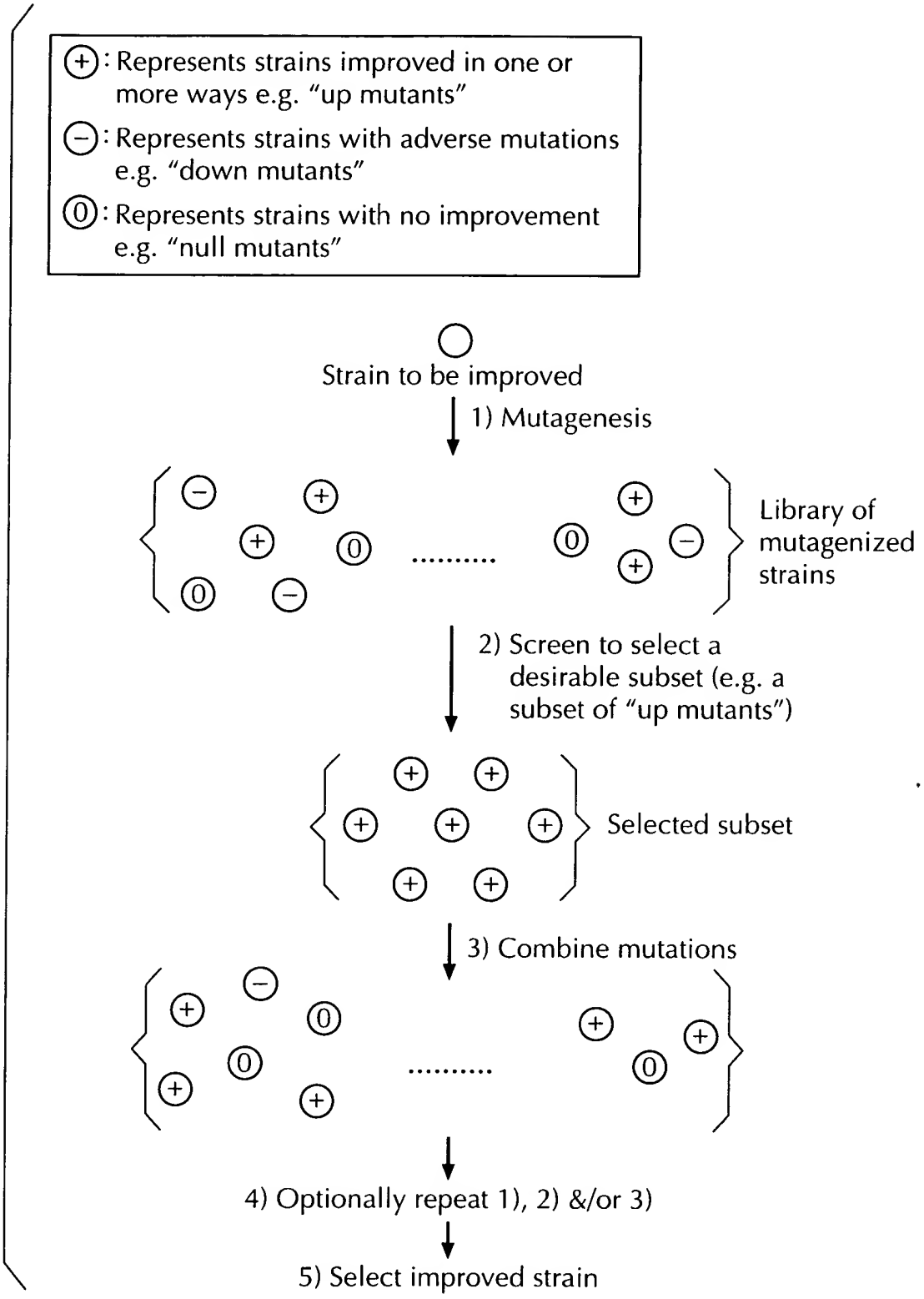

⊕: Represents strains improved in one or more ways e.g. "up mutants"
⊖: Represents strains with adverse mutations e.g. "down mutants"
⓪: Represents strains with no improvement e.g. "null mutants"
Strain to be improved
1) Mutagenesis
Library of mutagenized strains
2) Screen to select a desirable subset (e.g. a subset of "up mutants")
Selected subset
3) Combine mutations
4) Optionally repeat 1), 2) &/or 3)
5) Select improved strain

FIG. 22

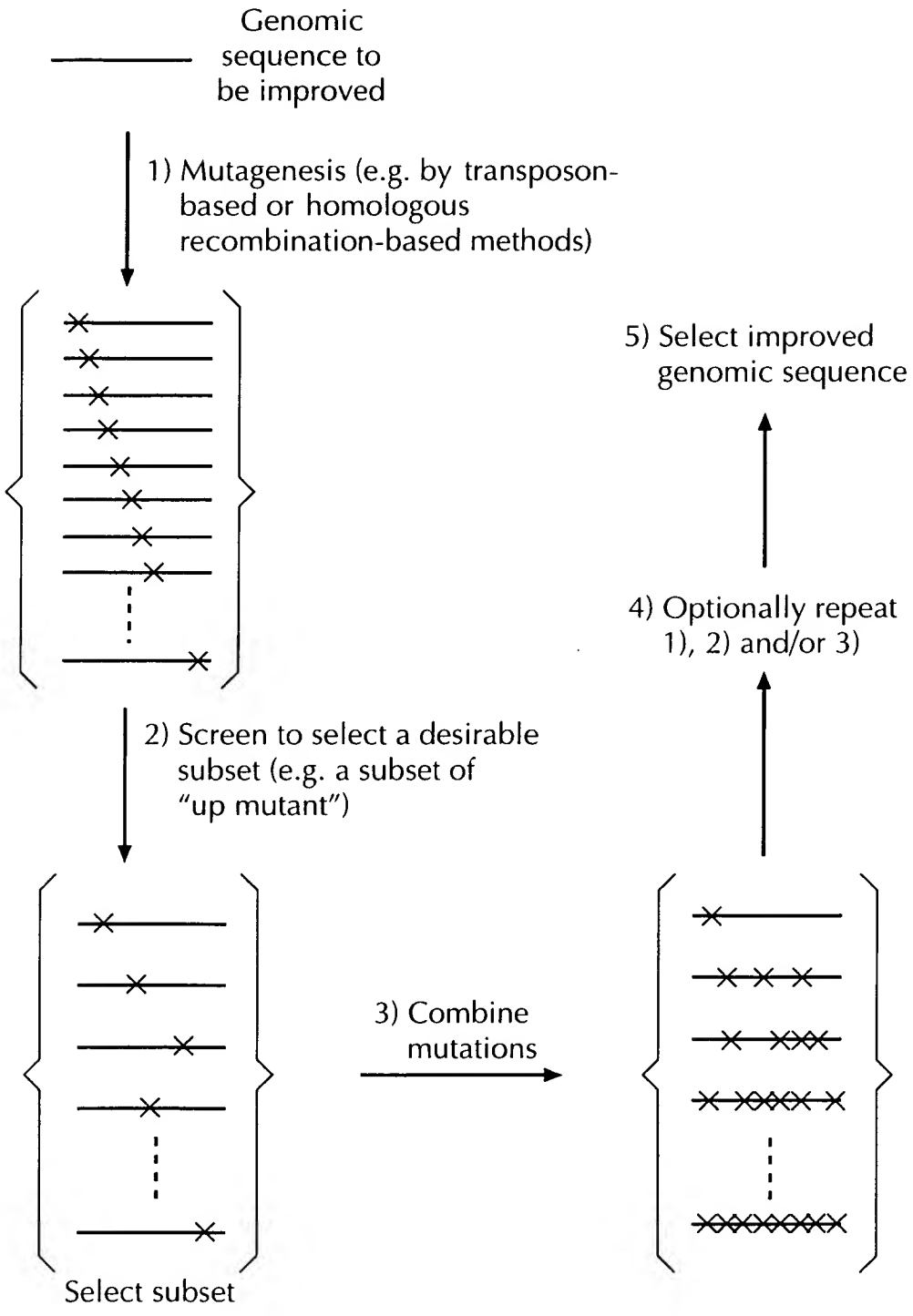

Genomic sequence to be improved
1) Mutagenesis (e.g. by transposon-based or homologous recombination-based methods)
2) Screen to select a desirable subset (e.g. a subset of "up mutant")
Select subset
3) Combine mutations
4) Optionally repeat 1), 2) and/or 3)
5) Select improved genomic sequence

FIG. 23

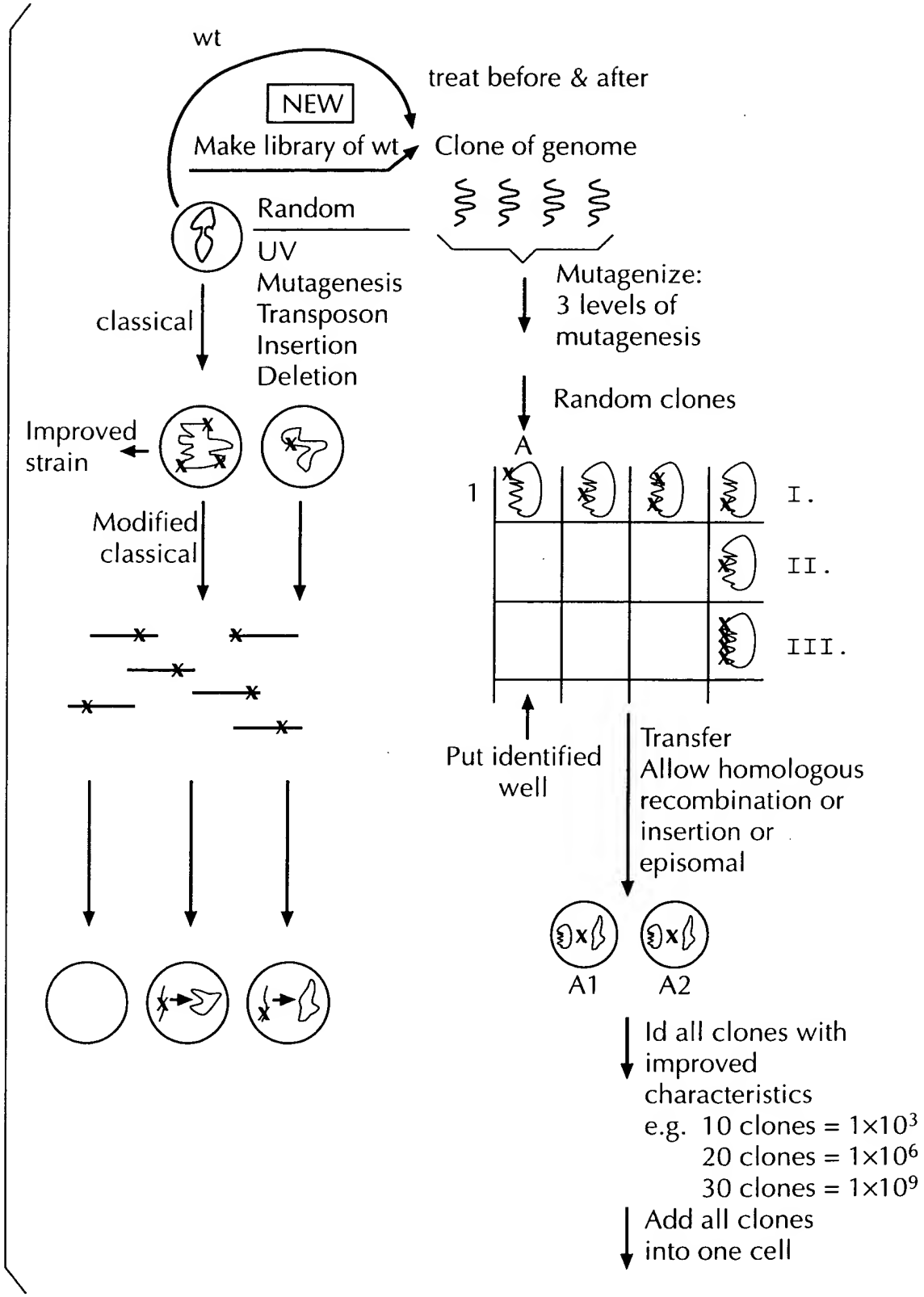
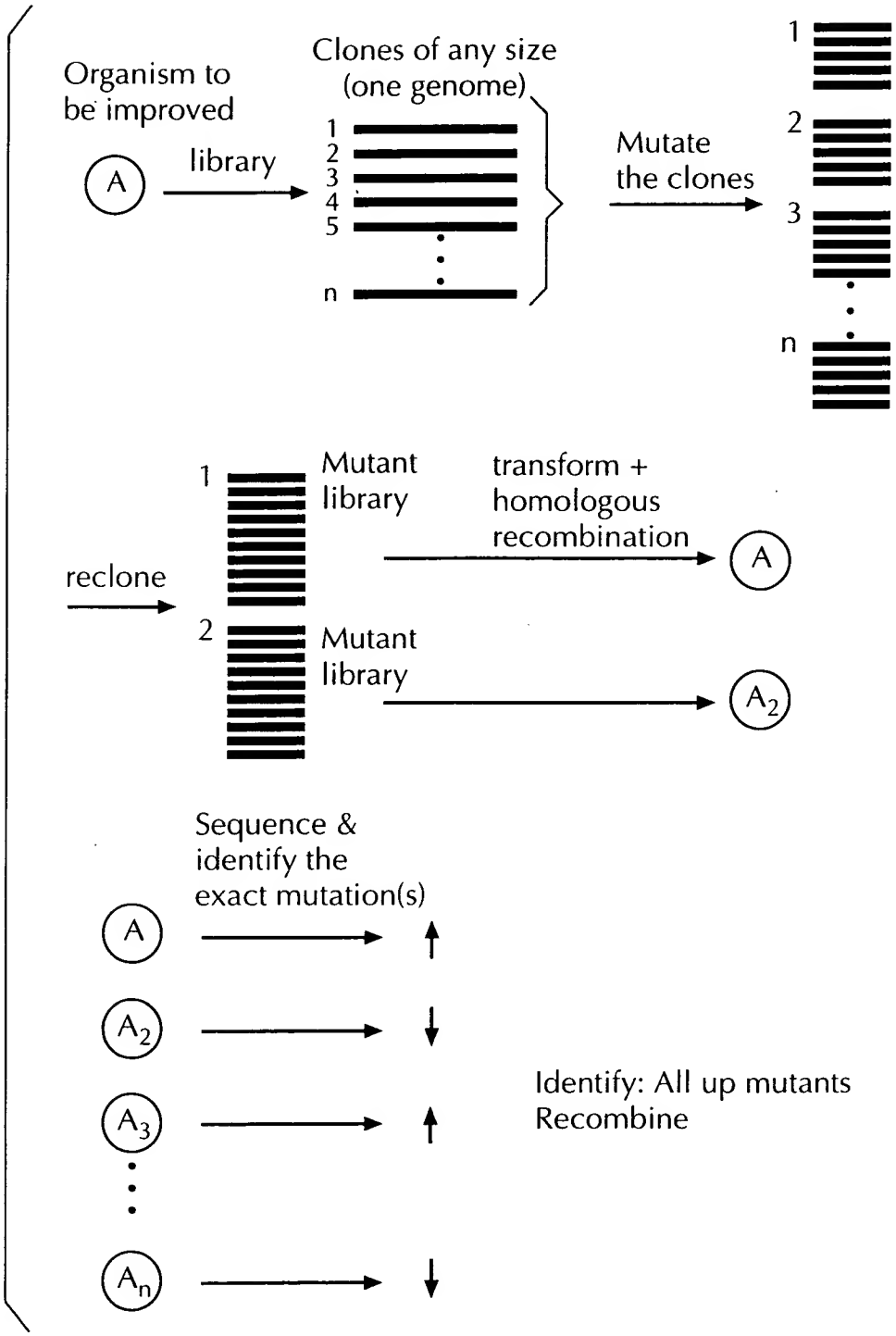

wt
NEW
Make library of wt
treat before & after
Clone of genome
Random
UV
Mutagenesis
Transposon
Insertion
Deletion
classical
Improved strain
Modified classical
Mutagenize:
3 levels of mutagenesis
Random clones
A
1
I.
II.
III.
Put identified well
Transfer
Allow homologous recombination or insertion or episomal
A1
A2
Id all clones with improved characteristics
e.g. 10 clones = 1×10^3
20 clones = 1×10^6
30 clones = 1×10^9
Add all clones into one cell

FIG. 24


Organism to be improved
A
library
Clones of any size (one genome)
1
2
3
4
5
n
Mutate the clones
1
2
3
n
reclone
1
2
Mutant library
Mutant library
transform + homologous recombination
A
A2
Sequence & identify the exact mutation(s)
A
A2
A3
An
Identify: All up mutants
Recombine